

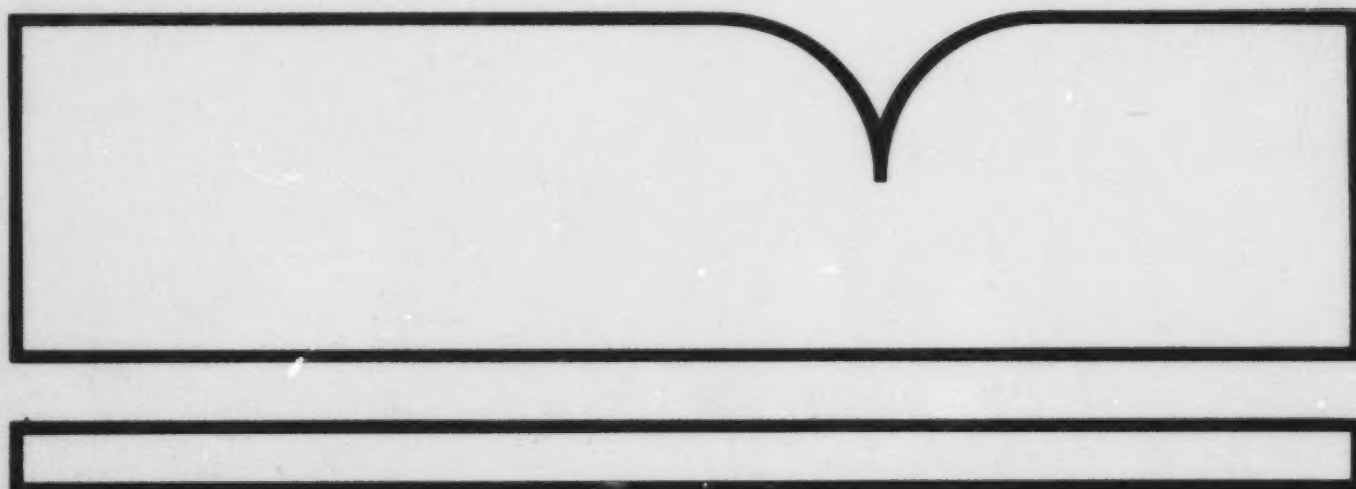
Current Status of Aneuploidy
Testing in Drosophila

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16. ABSTRACT A committee of scientists was established by the U.S. Environmental Protection Agency to appraise the current state of aneuploidy test methodology, to compile and analyze published data on the chemical induction of aneuploidy, and to provide guidance for additional test development and validation. The committee was organized into 9 working groups, representing different eukaryotic organisms ranging from fungi to whole mammals. The Environmental Mutagen, Carcinogen, and Teratogen Information Program at Oak Ridge National Laboratory identified and provided literature to the working groups. The 9 reports were prepared by the individual working groups. The reports include: (1) descriptions of the available testing approaches and general principles to be followed for protocol development, (2) criteria for evaluating data, and (3) recommendations for future test development and basic research. The evaluated results are presented in tables. For the positive and negative calls, the lowest effective dose tested or the highest ineffective dose tested, respectively, and the range of concentrations tested are provided. An overall summary report was prepared that summarizes the conclusions and recommendations of the 9 working groups and the chemical data base. It is hoped that these reports will provide a framework for further test development and validation of aneuploidy assays.		
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Current status of aneuploidy testing in *Drosophila* *

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* Report of the Aneuploidy Data Review Committee (Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC).

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Address reprint requests to the Environmental Mutagen, Carcinogen, and Teratogen Information Program, P.O. Box Y, Oak Ridge National Laboratory, Oak Ridge, TN 37830.

Summary

Based on the literature on file at EMIC, 181 papers contained material on aneuploidy testing. Initial screening rejected papers providing no data, no negative control and/or poorly designed genetic schemes; 67 papers representing tests of 76 compounds were reported on.

Statistical classifications were established as follows: (+) = a statistically significant difference at the 5% level between the treated and control frequencies; (-) = no significant difference at the 5% level when the number of offspring tested was sufficient to have identified an increase of 0.2% over the control with a power of 75%; I = inconclusive = (a) no significant difference at the 5% level but the number of offspring tested was below that necessary to detect an increase of 0.2% with a power of 75%; (b) the nature of apparent complete loss is undetermined; or (c) the nature of the germ cells sampled is not determined.

Of the 76 compounds analyzed, calls were made on 34 compounds. 17/34 compounds were positive for chromosome gain (11/34 for chromosome gain and chromosome loss, 6/34 for chromosome gain only). 17/34 compounds were negative for chromosome gain (11/34 for chromosome gain and chromosome loss and 6 for chromosome gain only).

Are any of the compounds found to induce aneuploidy specific for aneuploid induction? 7 of the compounds positive for chromosome gain were positive in one or more tests assaying for other genetic endpoints, and no reliable data exists regarding results in other tests for the remaining 10 compounds; accordingly, the answer to the question awaits further work.

(1) Introduction

(1.1) Background

At germ cell formation in standard *Drosophila* strains, homologous chromosomes usually pair at prophase I and separate at anaphase I; sister centromeres then separate at anaphase II and a chromatid is delivered to each of the 4 products. In the female, one of the outer products of a linear array of four becomes the functional egg; in the male, all products are functional. A large number of genes function in processes employed in the two sexes to ensure conjunction and separation of homologues (e.g., Sandler et al., 1968). The genetic control of cell division in males and females appears to be the same in mitotic cells (gonia and meiosis II); control of chromosome behavior at meiosis I is not (e.g., Baker and Carpenter, 1972). The differences are related primarily to the absence of recombination in the male (e.g., Cooper, 1950). Comparison with mammalian meiosis suggests that meiosis I in male *Drosophila* is aberrant in this respect.

Nondisjunction of the sex chromosomes in XX females gives rise to XX and nullo-X eggs. Nondisjunction in XY males produces XY and nullo-XY

gametes. The recovered frequency of diplo sex chromosome gametes (chromosome gain) is about 0.05%; nullo gametes (chromosome loss) are about 3-8 times more frequent, reflecting chromosome loss independent of nondisjunction. The frequency of spontaneous nondisjunction of the autosomes appears to be similar to that of sex chromosomes. Simultaneous nondisjunction of sex chromosomes and autosomes occurs with a frequency substantially greater than would be expected if they occurred independently; this observation holds both for males and females, suggesting cellular conditions that promote nondisjunction of more than one chromosome pair (see Zimmering, 1975, for review of spontaneous nondisjunction in *Drosophila*).

Nondisjunction may arise by (a) nonconjunction leading to failure of homologues to separate at anaphase I, (b) the failure of sister chromatids to separate at anaphase II following normal segregation of homologues at anaphase I, or (c) precocious separation of centromeres at anaphase I (Davis, 1971; Mason, 1976). Genetic analysis of spontaneously arising exceptional daughters from ordinary XX females suggests that (a) virtually all nondisjunction results from the failure of the reduction division, (b) exceptional females may arise

from exchange tetrads, and (c) some 40% of all exceptional females are equational exceptions in that they are homozygous for some part of the chromosome. The frequency of homozygosis for a given gene is proportional to the distance of the gene from the centromere (Merriam and Frost, 1964).

Factors affecting meiotic segregation include the presence of an extra chromosome or chromosome element (Bridges, 1916; Lindsley and Sandler, 1965), inversion heterozygosity especially where X and an autosome are heterozygously inverted (Cooper et al., 1955), temperature (Tokunaga, 1970; Grell, 1971), X-rays (Mavor, 1922; Traut, 1971), and meiotic mutants. Studies on meiotic mutants have provided critical information on the delineation of control points at which the wild-type alleles of these mutations are presumed to act with respect to known genetic landmarks of meiosis, including processes related to pairing, recombination, segregation of homologues at meiosis I, and separation of sister chromatids at meiosis II (Baker and Carpenter, 1972).

Investigations on the mechanism of X-ray-induced nondisjunction in the *Drosophila* female suggest that nondisjunction occurs when induced chromatid-type heterologous interchanges in stage 7 oocytes are followed by separation of interchange heterologues and random assortment of the normal pairing partners with respect to the homologous elements involved in the interchange (Parker, 1970). The possibility suggests itself that the same mechanism may turn out to hold for some clastogenic chemical agents.

Finally, a recent Environmental Protection Agency (EPA) Gene-Tox report (Valencia et al., 1984) provided the first comprehensive analysis of papers dealing with chemically induced aneuploidy in *Drosophila*, a listing of the compounds tested, and judgments regarding positive/negative responses. Although the present study reviews much of the material in that report, it also expands the data base, provides the rationale for a more reasonable statistical approach in the assessment of the aneuploidy-inducing capability of chemical agents, and describes several new aneuploidy-detecting systems under development.

(1.2) Literature review

Based on the literature on file at the Environmental Mutagen Information Center, 181 papers were identified as containing material on aneuploid testing. Included were published papers, notes from *Drosophila* Information Service, abstracts, and government reports. Initial screening by the panel rejected papers providing calls but no data, no negative controls, and/or poorly designed genetic detection schemes. A total of 67 papers representing tests of 76 compounds are reported.

(2) Test description

(2.1) Genetic schemes employed

Two basic test protocols have been employed to monitor induced sex chromosome aneuploidy in *Drosophila*. The most often used protocol involves male treatment. As an illustration, males bearing an X chromosome marked with *y* (yellow body color) and Y chromosome markers derived from the X chromosome, typically $B^S Y y^+$ (B^S = dominant *Bar eye of Stone*; y^+ = wild-type allele of *y*), are treated as larvae or adults, and mated with $y w^a$ females (yellow body, apricot eye color). The regular offspring from this cross are phenotypically *y* females ($y/y w^a$) and $w^a B^S$ males ($y w^a/B^S Y y^+$). Apparent complete loss of either paternal sex chromosome results in the recovery of $y w^a$ males, while induced nondisjunction of the X and Y can be detected by the recovery of B^S females ($y/y w^a/B^S Y y^+$).

Also recoverable are males resulting from partial loss. These males carry one, but not both, of the Y chromosome markers: loss of B^S (which marks the long arm of the Y chromosome) can be detected by the recovery of w^a males in the F_1 , while losses of y^+ from the short arm can be recovered as $y w^a B^S$ males. Partial losses likely arise from 2-break events (e.g., induced interchange between the arms of the Y chromosome, a Y-autosome translocation, or a deletion event).

While partial loss events are of considerable importance in assessing the clastogenic potential of a test chemical, such data do not constitute a valid assay for aneuploidy. Similarly, reports on analysis of so-called complete chromosome loss

induced by X-rays (Maddern and Leigh, 1976) or chemicals (Zimmering and Kammermeyer, 1985) suggest that a substantial proportion are gross deletions of the X or Y chromosome. Based on these considerations, this panel viewed chromosome loss data in the absence of corroborating data on chromosome gain as inconclusive.

A second type of assay system, one which was less frequently used than the male-treated protocol in the papers reviewed here, employs treatment of larval or adult females. Typically, the female bears structurally normal and identical X chromosomes marked with, for example, *y*. When such females are mated with males of the appropriate composition such as *w/Y* (*w* = white eye color), the regular offspring are wild-type females (*y/w*) and *y* males (*y/Y*). Loss of a maternal X chromosome can be detected by the recovery of a patroclinous *w* male (*w/0*), while nondisjunction of the maternal Xs is expected to generate both matroclinous *y* females (*y/y/Y*) and patroclinous *w* males (*w/0*). While one would expect that the frequency of exceptional females would not exceed that of exceptional males, there are, in fact, curious exceptions to this rule (Ramel and Magnusson, 1969) which are likely derived from sampling of treated oogonia (Mason, 1976). In view of results from X-ray-induced nondisjunction (Section 1), it is important to bear in mind that in immature oocytes (stage 7 and earlier), chromosome interchanges can result in the recovery of viable, aneuploid gametes (e.g., Parker, 1970).

(2.2) Suggested protocols

(2.2.1) Gender

It is the view of this panel that the preferred protocol for the detection of induced sex chromosome aneuploidy would likely be one which employs treatment of females. The principal argument against the use of male-treatment protocols is the absence of meiotic recombination in *Drosophila* males as indicated in Section 1. The lack of a recombination in spermatocytes militates against the detection of chemicals which exert their effects by interfering with this process. Since most organisms, including humans, exhibit meiotic recombination, the use of *Drosophila* males makes more precarious the extrapolation to other

organisms. Nonetheless, it should be pointed out that there is no compelling experimental evidence at this time to justify exclusion of the male as a test vehicle.

(2.2.2) Route of exposure

The simplest procedure for the treatment of either larvae or adults is feeding. Whether this procedure, or an alternative exposure route, is employed will be determined by factors including chemical solubility, stability, and palatability. It is suggested that where there is a published precedent for one method of exposure (e.g., a positive result for a sex-linked recessive lethal test), that the same procedure be used. Where there is no precedent, it is expedient to try feeding and then, if feeding proves ineffective, proceed to other treatment (injection, topical application, inhalation).

(2.2.3) Solvent

The choice of solvent is determined, in part, by the solubility properties of the test chemical. Water-soluble compounds are readily dispersed in sucrose solutions for feeding or in saline for injection of adults. Other chemicals may require the addition of an organic solvent; in these cases, care must be exercised since some of the frequently used solvents have undesirable side effects. For example, DMSO apparently depresses the activity of some microsomal enzymes that may affect the metabolism of indirect-acting mutagens (i.e., DEN) which require metabolic activation (e.g., Valencia et al., 1984). It may be possible to achieve adequate mixing of the test chemical with *Drosophila* food without resorting to use of organic solvents.

In preparing aqueous solutions it is important that water of the highest purity possible be used, preferably deionized, glass-distilled water. The use of tap water is strongly discouraged. In addition, exposures should take place in clean glassware containers, and glass-fiber filters should be used for adult feedings. The use of plastics is discouraged because of the possibility of interaction between the test chemical and the plastic.

(2.2.4) Toxicity levels

It is suggested that toxicity determinations be made with each test chemical and that treatments

be made with one or more dose levels which produce measurable toxicity. The advantage of employing doses known to induce toxicity is 2-fold. This ensures that the animals have ingested the chemical, facilitates interexperiment and interlaboratory comparison. When a particular batch of chemical is used repeatedly over a period of time, it is important to repeat the toxicity tests regularly to assess the stability of the compound.

It is not infrequently observed that chemical doses which are non-lethal during the treatment period (typically 24–72 h in the case of adult feeding) nonetheless produce a delayed effect; substantial numbers of animals may die several days after treatment. Delayed effects are of particular importance when brooding is employed as the germ cells of interest may not be sampled until a week or so after treatment. Likewise, delays in the development of treated larvae have also been reported (Zettle and Murnik, 1973). Dosages may have to be adjusted to permit efficient sampling of the desired germ cells.

It is often the case that doses of chemical that produce modest lethality have profound effects on fertility. Moreover, meiocytes appear to be especially sensitive to sterilisation. As these are the cells of greatest interest, it is important to adjust dose levels to permit adequate sampling of treated spermatocytes and oocytes.

(2.2.5) *Palatability*

Palatability tests are typically carried out in conjunction with the toxicity trials described above. There are several convenient measures of ingestion; the simplest is direct observation of the feeding behavior of the adults or larvae. Particularly noxious chemicals may elicit avoidance behaviors (i.e., adults refuse to land on the chemical-soaked surface or larvae quickly move off the food surface and onto the sides of the glass treatment vessel). More sophisticated measures of ingestion include addition of a neutral dye to the chemical solution during the palatability trials (Maroni and Stamey, 1983) and the use of radiolabeled sucrose for those compounds that are soluble in aqueous solution (Ayaki et al., 1984). In some cases, flies can be forced to feed on unpalatable compounds by leaving the animals in the treatment vessels for several days. Under these conditions, flies that do not feed

die of desiccation. Palatability is not an issue during injection.

(2.2.6) *Dose*

As mentioned above, doses should be selected which produce measurable toxicity levels. The choice of toxicity level is flexible; for example, several laboratories employ LD_{30} and LD_3 dose levels. The ultimate criterion is biological effectiveness. In some cases, very high LDs may be required to produce statistically significant results, although the biological significance of tests run at high LDs would be called into question. Alternatively, different routes of administration may be employed following initial failure to detect an effect. Whatever the treatment strategy, multiple toxic doses should be employed where possible.

It should also be noted that the concentration of the chemical presented to the animal is almost certainly not the concentration that reaches the gonads, nor is it always the case that the biological response is linearly related to the dose.

(2.2.7) *Mating and brooding*

Where practical, it is suggested that individual treated animals be provided with several mates (e.g., 1 treated male with 3 virgin females). The advantage of employing single male (or single treated female) matings is that this permits one to identify clusters of exceptional offspring derived from a single treated animal. Depending on the developmental stage treated and the brood(s) in which clusters are sampled, one is able to identify presumptive spontaneous events that are unrelated to chemical treatment. Against these advantages must be weighed limitations in personnel and/or materials necessary to execute pair matings. An acceptable alternative may be to employ small mass matings; however, the mating pressure (number of mates per treated animal) should be maintained at 3 or 4 to 1.

The brooding schedule will be determined principally by the stage(s) at which treatment was begun. The goal is to sample treated meiocytes. Thus, treated adult males will have to be brooded in a series of 2–3-day broods through days 6–9 to ensure sampling of treated spermatocytes. In the case of treated larvae, the length (in days) per brood and the number of broods would be dictated by the larval stage selected for treatment.

(3) Data collection and interpretation

Data from the aneuploidy test should be presented in sufficient detail to permit a thorough analysis of how the experiment was executed and the results obtained. Where brooding was done, the results from the individual broods should be presented; pooling of data from several broods should be done only when appropriate statistical tests fail to show heterogeneity among the pooled broods. Where male treatments have been employed, complete and partial losses should be presented separately. The number and size of clusters should be recorded and appropriate statistics applied (Owen, 1962). Results should be stated as the frequency of exceptional offspring per total number of offspring.

(3.1) Controls

Concurrent negative controls are an absolute requirement for aneuploidy tests. This is true because the data base, especially for females, is relatively modest and the possibility of inhomogeneity and its statistical treatment have yet to be explored.

Positive controls should be run on a regular basis, although not necessarily with very chemical test. A protocol in which a positive control is run with every fifth experiment is entirely acceptable. Several model compounds suggest themselves as suitable positive controls, including colchicine (or colcemid) and 5-fluorodeoxyuridine (Held, 1982; Traut, 1978).

(3.2) Sample size determination

The appropriate experimental sample size is determined by several factors. The choice of statistical tests is one of the most important and least considered decisions that an experimenter must make. Margolin et al. (1983) have argued for the use of a test based on the normal approximation to the binomial distribution when analyzing binomial data in which the frequency of the positive (i.e., aneuploid) class is less than 1%. This test is more powerful and requires a smaller sample size than the conditional binomial test tabulated by Kastenbaum and Bowman (1970). It should be

pointed out that the Kastenbaum-Bowman tables were not designed to be used for sample size determinations and should not be used for this purpose.

A few factors that need to be considered when determining sample size are widely accepted or have been discussed recently. The significance level, for example, is routinely set at 5%. When using concurrent negative controls, equal sample sizes in the treated and control series are recommended. This approach has been shown to be most efficient (Lehman, 1959; Berchtold, 1975). No strong arguments have been made concerning the size of the induced increase in the frequency that an experiment should detect. The present panel has adopted the recommendation of Lee et al. (1983) to examine the data for an induced increase of 0.2% over the spontaneous control.

The underlying spontaneous frequency is critical in sample size determinations. This frequency is strain dependent and may be influenced by experimental conditions. In addition, it is often difficult to determine the underlying frequency, especially when final protocols have not been established. Empirical determinations are merely approximations of the true frequency. Several independent determinations must, therefore, be made before accurate estimates can be achieved. Estimates of overall spontaneous aneuploidy fre-

TABLE 1
MINIMUM SAMPLE SIZES REQUIRED IN EACH OF THE TREATED AND CONTROL SERIES TO DETECT AN INCREASE OF 0.2% IN THE ANEUPLOID FREQUENCY USING THE NORMAL TEST AT THE 5% SIGNIFICANCE LEVEL

Control (%)	Power			
	0.50	0.75	0.90	0.95
0.00	1338	1961	2662	3148
0.05	1938	3561	5661	7157
0.10	2557	5124	8043	10136
0.15	3327	6499	10330	12998
0.20	4049	7921	12466	15746
0.25	4689	9259	14701	18571
0.30	5404	10557	16880	21342
0.35	6082	12008	19070	24059
0.40	6721	13342	21204	26799
0.45	7349	14731	23386	29529
0.50	8072	16029	25548	32237

TABLE 2
COMPOUNDS EVALUATED FOR THE INDUCTION OF ANEUPLOIDY

Chemical (CAS No.)	References	Treatment parameters					End point ^f		Consensus	
		Sex ^a	Stage ^b	Route ^c	Cell ^d	Concentration ^e	Gain	Loss	Gain	Loss
Mitomycin-C (50-07-7)	Walker and Williamson, 1975	F	A	F	Cyt	600 µg/ml	-	I ²	-	I
	Ostertag and Haake, 1966	F	L	F	Cyt, Gon	600 µg/ml	-	I ²	-	I
<i>p,p'</i> -DDT (50-29-3)	Clark, 1974	M	A	F	Cyt, Gon	NI ^f	+	+	+	+
		M	A	F	Spm, Tid	NI ^f		I ²		
Thalidomide (50-35-1)	Luning and Jernudd, 1967	M	L	F	Cyt, Gon	9%	-	-	-	-
Actinomycin-D (50-76-0)	Felix and Rodriguez, 1968	F	A	IJ	Cyt	0.1 mg/ml	+	+	+	+
5-Fluorodeoxyuridine (50-91-9)	Traut, 1978	F	A	F	Cyt	12.5 µg/ml	+	+	+	+
		F	A	F	Gon	12.5 µg/ml	+	+		
Urethane (51-79-6)	Knaap and Kramers, 1982 Mittler et al., 1966	M	A	F	Spm	1%		I ²	-	I
		M	A	IJ	Cyt	7 mg/ml	-	I ²		
		M	A	IJ	Spm	5-7 mg/ml		I ²		
Caffeine (58-08-2)	Zettle and Murnik, 1973	M	L	F	Cyt, Gon	0.5%	-	+	+	+
	Zimmering and Osgood, 1975	F	A	F	Cyt	0.2-1%		I ¹		
	Nothel and Abdalla, 1982	F	A	F	Cyt	0.2%		I ¹		
	Clark and Clark, 1968	F	L	F	Cyt, Gon	10 mM	-	+		
		F	A	F	Cyt, Gon	10 mM	-	-		
	Mittler et al., 1967a	M	L	F	Cyt, Gon	10 mM	-	+		
		M	L	F	Cyt, Gon	0.123%	+	I ²		
	Mittler et al., 1967b	M	A	F	Spm, Tid	0.123%		I ²		
		M	A	F	Cyt, Gon	0.123%	+	I ²		
	Ostertag and Haake, 1966	F	L	F	Cyt, Gon	10 mg/ml	-	+		
Amitrole (61-82-5)	Laamenen et al., 1976	F	A	F	Cyt, Gon	10 ppm	-	-	-	-
Dimethyl sulfoxide (67-68-5)	Traut, 1983	F	A	F	Cyt, Gon	2%	-	-	-	-
Triaziquone (68-76-8)	Buchi and Burki, 1975	M	A	F	Spm	0.01 mM		I ²	-	-
		F	A	F	Cyt	0.01 mM	-	-		
	Vogel, 1973	M	A	F	Spm	0.01 mM		I ²		
		M	A	F	Spm	0.02-0.04 mM		I ²		
MNNG (70-25-7)	Browning, 1969	M	A	IJ	Gon	NI ^h	+	-	+	-
		M	A	IJ	Cyt	NI ^h	I ¹	I ¹		
		M	A	IJ	Spm, Tid	NI ^h		I ²		
Glutethimide (77-21-4)	Luning, 1966	M	L	F	Cyt, Gon	2500 ppm	-	-	-	-
Acenaphthene (83-32-9)	Zivin, 1946	F	L	F	Cyt, Gon	NI ⁱ	+	+	+	+
Proflavine (92-62-6)	Ostertag and Haake, 1966	F	L	F	Cyt, Gon	100 mg/ml	+	+	+	+
1,2-Dichloroethane (107-06-2)	Shakarnis, 1969	F	A	IH	Cyt, Gon	4.7 ppm	+	+	+	+

TABLE 2 (continued)

Chemical (CAS No.)	References	Treatment parameters					End point ^f		Consensus	
		Sex ^a	Stage ^b	Route ^c	Cell ^d	Concentration ^e	Gain	Loss	Gain	Loss
Simazine (122-34-9)	Murnik and Nash, 1977	M	L	F	Cyt, Gon	0.6%	-	-	-	-
2-Methoxyethyl- mercury chloride (123-88-6)	Ramel and Magnusson, 1969	F	A	F	Cyt, Gon	20 µg/ml	-	I ²	-	I
Sodium cyclamate (139-05-9)	Felix and de la Rosa, 1971	F	A	F	Cyt	100 mg/ml	+	+	+	+
Halothane (151-67-7)	Clements and Todd, 1981	F	A	F	Cyt	0.34%	+	+	+	+
Heliotrine (303-33-3)	Brink, 1969	M	A	IJ	Cyt, Gon	1 mM	-	I ²	-	I
Azathioprine (446-86-6)	Clark, 1975	M	A	F	Gon	NI ⁸	+	+	+	+
		M	A	F	Cyt	NI ⁸	-	-	-	-
		M	A	F	Spm, Tid	NI ⁸	-	I ²	-	-
Colcemid (477-30-5)	Held, 1982	F	L	F	Cyt, Gon	2 µg/ml	+	-	+	-
		M	L	F	Cyt, Gon	4 µg/ml	-	-	-	-
Vinblastine (865-21-4)	Sorsa et al., 1980	F	A	F	Cyt, Gon	100 ppm	+	-	+	-
		M	A	F	NI	100 ppm	I ³	I ^{2,3}	-	-
Methylmercury hydroxide (1184-57-2)	Ramel and Magnusson, 1979 Ramel and Magnusson, 1969	F	L	F	Cyt, Gon	0.25 µg/ml	+	-	+	-
		F	A	F	Cyt, Gon	0.25 µg/ml	+	-	-	-
		M	A	F	NI	0.25-5 µg/ml	I ³	I ³	-	-
		F	L	F	Cyt, Gon	0.25 µg/ml	+	-	-	-
		M	L	F	Cyt, Gon	0.25 µg/ml	-	-	-	-
Atrazine (1912-24-9)	Murnik and Nash, 1977	M	L	F	Cyt, Gon	1 µg/ml	-	I ²	-	I
Sodium fluoride (7681-49-4)	Vogel, 1973	F	A	F	Cyt	12 mM	-	-	-	-
		M	A	F	Spm	12 mM	-	I ²	-	-
Clophen A50 (8068-44-8)	Nilsson and Ramel, 1974	M	L	F	Cyt, Gon	200 µg/ml	+	-	+	-
		M	A	F	NI	200 mg/ml	I ³	I ³	-	-
Bleomycin (11056-06-7)	Traut, 1980	F	A	F	Cyt	50 µg/ml	+	+	+	+
<i>cis</i> -Dichlorodiamine- platinum II (15663-27-1)	Brodberg et al., 1983	M	A	IJ	Spm, Tid	0.2 mM	-	I ²	-	I
		M	A	IJ	Cyt	0.2 mM	-	I ⁴	-	-
		F	A	IJ	Cyt	0.2 mM	I ¹	I ¹	-	-
Benomyl (17804-35-2)	Lamb and Lilly, 1980	M	A	F	Spm, Tid	1 mg/ml	-	I ²	-	-
		M	A	F	Cyt, Gon	1 mg/ml	-	-	-	-
Fusarium T-2 toxin (21259-20-1)	Sorsa et al., 1980	F	A	F	Cyt, Gon	250 ppm	-	-	-	-
		M	A	F	NI	20-250 ppm	I ³	I ^{2,3}	-	-
Cyanazine (21725-46-2)	Murnik and Nash, 1977	M	L	F	Cyt, Gon	0.01%	-	-	-	-

TABLE 2 (continued)

Chemical (CAS No.)	References	Treatment parameters					End point ^f		Consensus	
		Sex ^a	Stage ^b	Route ^c	Cell ^d	Concentration ^e	Gain	Loss	Gain	Loss
Satratoxin-H (53126-64-0)	Sorsa et al., 1980	M	A	F	IN	250 ppm	I ^{1,3}	I ^{1,2,3}	+	I
		F	A	F	Cyt, Gon	250 ppm	+	I ¹		
Clophen A30 (54991-93-4)	Nilsson and Ramel, 1974	M	L	F	Cyt, Gon	250 µg/ml	-	-	-	-

^a M, male; F, female.^b Developmental stage treated: A, adult; L, larva.^c Exposure route: F, feed; IJ, injection; IH, inhalation.^d Cell type treated: Spm, spermatozoa; Tid, spermatid; Cyt, spermatocyte or oocyte; Gon, spermatogonia or oogonia; All, all germ cell stage; NI, not indicated or no distinction possible.^e In most studies a single concentration was used. If the result is positive, the concentration given is the lowest effective dose. If the result is negative the concentration given is the highest ineffective dose.^f +, positive; -, negative; I, inconclusive for one of the following reasons: ¹ inadequate sample size to determine a negative, ² nature of apparent complete loss undetermined, ³ germ cells not determined, ⁴ inadequate sample size to determine a positive.^g Placed on surface of food.^h 'Saturated solution'.ⁱ Conclusions reached after summing the data from experiments using various concentrations.

quencies for the X chromosome in ordinary XX females cluster around 0.05–0.1%, although there is some variability.

The power of the proposed test (i.e., the ability to detect aneuploidy-inducing chemicals when they are tested) is also important in determining the appropriate sample size. Table 1 shows the minimum required sample sizes as a function of the power of the test, assuming that (1) the test is designed to detect an incremental increase 0.2%, at the 5% significance level, using the normal test described by Margolin et al. (1983) and (2) equal sample sizes in the treated and control series. Because the assays for aneuploidy involve screening F_1 progeny, it is reasonable to expect to detect an aneuploidy-inducing chemical with a power of 95%. If multiple doses are tested simultaneously, it might be reasonable to test each concentration at a power of 90%.

(4) Interpretation of data

(4.1) Presentation of data

The results of the survey of chemically induced aneuploidy are shown in Tables 2 and 3. Chemicals on which it was reasonable to make a decision about ability to induce aneuploidy are listed

in Table 2. Chemicals for which data were not sufficient to make such a decision are listed in Table 3. The criteria used to evaluate the data are given below. It is known that procedural details can affect results in mutagenicity experiments. Therefore, the route of administration of the chemical, the concentration, the sex and developmental stage of the treated individual, and the developmental stage of the treated germ cells are all indicated in the tables. Where appropriate, the reasons that data were found unacceptable for making a judgment are also given.

(4.2) Criteria for acceptability of data

In the previous section, desirable protocols for monitoring chemically induced aneuploidy are outlined. Only a small fraction of the data in the literature were obtained using appropriate experimental protocols for monitoring chromosome segregation. For purposes of the present review, it was necessary, therefore, to apply to these papers somewhat more relaxed procedural criteria than those recommended for future testing. As indicated in Section 1, papers were not considered for review if control data were lacking or if the experimental protocol was not outlined in sufficient detail to determine how the experiment was per-

TABLE 3

COMPOUNDS THAT COULD NOT BE CLASSIFIED AS POSITIVE OR NEGATIVE IN ANEUPLOIDY ASSAYS IN DROSOPHILA

Chemical (CAS No.)	References	Treatment parameters					Comments ^c
		Sex ^a	Stage ^b	Route ^c	Cell ^d	Concentration	
Cyclophosphamide (50-18-0)	Vogel, 1975	M	A	F	Spm	10 mM	2
	Zimmering, 1982c	M	A	F	Spm	10 mM	2
Lysergic acid diethylamide (50-37-3)	Browning, 1968	M	A	IJ	NI	10 mg/ml	2, 3
Sorbitol (50-70-4)	Abbott and Bowman, 1976	M	A	F	NI	5%	1, 2, 3
Triethylene melamine (51-18-3)	Ikebuchi and Teranishi, 1981	M	A	F	Spm	50-100 μ M	2
	Snyder and Oster, 1964	M	A	IJ	Spm	0.2 mM	2
Diethylnitrosamine (55-18-5)	Vogel and Leigh, 1975	M	A	F	Spm	5 mM	2
	Vogel and Natarajan, 1979	M	A	F	Tid	0.1 mM	2
	Zimmering et al., 1981	M	A	F	Spm	5-10 mM	2
	Zimmering, 1981; Zimmering and Kammermeyer, 1983	M	A	F	Spm	10 mM	2
Adenosine triphosphate (56-65-5)	Mittler and U., 1966	M	A	IJ	Spm	5 mg/ml	1, 2
		M	A	IJ	Cyt, Gon	5 mg/ml	1, 2
Meprobamate (57-53-4)	Luers et al., 1974	M	A	F	Spm	4.6 mM	2
β -Propiolactone (57-57-8)	Zimmering, 1983b	M	A	F	Spm	7.5 mg/ml	2
Ethyl methanesulfonate (62-50-0)	Sobels, 1979	F	A	F	Cyt, Gon	12.5 mM	1
	Vogel and Natarajan, 1979	M	A	F	Spm, Tid	0.5 mM	2
	Ikebuchi and Teranishi, 1981	M	A	F	Spm	2.5 mM	2
	Zimmering, 1983b	M	A	F	Spm	3.9 mM	2
Dimethylnitrosamine (62-75-9)	Vogel and Natarajan, 1979	M	A	F	Spm, Tid	2-10 mM	2
	Zimmering et al., 1980	M	A	F	Spm	2.5 mM	2
	Zimmering, 1982b	M	A	F	Spm	0.01-0.05 mM	2
Diethyl sulfate (64-67-5)	Zimmering, 1983b	M	A	F	Spm	0.75%	2
	Abraham et al., 1979	M	A	IH	Spm, Tid	NI ^f	1, 2
		M	A	IH	Cyt, Gon	NI ^f	1, 2
Colchicine (64-86-8)	Mittler et al., 1966	M	A	IJ	Spm, Tid	0.2 mg/ml	1, 2
		M	A	IJ	Cyt	0.2 mg/ml	1
Methyl methanesulfonate (66-27-3)	Vogel and Leigh, 1975	M	A	F	Spm	0.5 mM	2
	Vogel and Natarajan, 1979	M	A	F	Spm, Tid	1-4 mM	2
	Zimmering and Kammermeyer, 1980	M	A	F	Spm	0.5 mM	2
	Zimmering, 1982b	M	A	F	Spm	0.5 mM	2
Vinyl chloride (75-01-4)	Verburt and Vogel, 1977	M	A	IH	Spm, Tid	30 mg/ml	2
Dimethyl sulfate (77-78-1)	Vogel and Natarajan, 1979	M	A	F	Spm	5 mM	2
1,2-Dibromo-3-chloro- propane (96-12-8)	Zimmering, 1983a	M	A	F	Spm	0.2 mg/ml	2

TABLE 3 (continued)

Chemical (CAS No.)	References	Treatment parameters					Comments ^c
		Sex ^a	Stage ^b	Route ^c	Cell ^d	Concentration	
2,3-Dibromopropanol (96-13-9)	Zimmering, 1982a	M	A	F	Spm	0.5 mg/ml	2
N-Nitrosopiperidine (100-75-4)	Zimmering, 1982d	M	A	F	Spm	5-10 mM	2
Allyl glycidyl ether (106-92-3)	Zimmering, 1983b	M	A	F	Spm	5.5 mg/ml	2
Butylated hydroxytoluene (128-37-0)	Sankaranarayanan, 1983 Prasad and Kamra, 1974	F	L	F	Cyt	0.5%	1
		M	A	IJ	Spm	10 ppm	2
Lasiocarpine (303-34-4)	Zimmering, 1983b	M	A	IJ	Spm	1 mg/ml	2
Procarbazine (366-70-1)	Blijleven and Vogel, 1977	M	A	F	Spm, Tid	30 mM	2
	Zimmering and Deitemeyer, 1981	M	A	F	Spm	1-5 mM	2
	Zimmering and Kammermeyer, 1980	M	A	F	Spm	10 mM	2
	Zimmering, 1981	M	A	F	Spm	5 mM	2
	Zimmering, 1983c	M	A	F	Spm	5 mM	2
	Zimmering and Kammermeyer, 1983	M	A	F	Spm	5 mM	2
N-Methyl-N-nitrosourea (684-93-5)	Vogel and Natarajan, 1979	M	A	F	Tid	1 mM	2
	Zimmering, 1983b	M	A	F	Spm	0.5 mM	2
Dihydroxyurea (686-68-0)	Fahmy and Fahmy, 1970	M	A	IJ	NI	50-100 mM	3
Ethyl nitrosourea (759-73-9)	Zimmering, 1983b	M	A	F	Spm	2 mM	2
	Vogel and Natarajan, 1979	M	A	F	Spm, Tid	2.5-10.2 mM	2
Isopropyl methanesulfonate (926-06-7)	Vogel and Natarajan, 1979	M	A	F	Spm	10 mM	2
1,2:3,4-Diepoxybutane (1464-53-5)	Zimmering, 1983b	M	A	F	Spm	1.25 mM	2
Dichloroacetonitrile (3018-12-0)	Zimmering, 1983b	M	A	F	Spm	0.25 mg/ml	2
Isophosphamide (3778-73-2)	Vogel, 1975	M	A	F	Spm	10 mM	2
Quinacrine mustard (4213-45-0)	Snyder and Oster, 1964	M	A	IJ	Spm	2 mM	2
2,3,5,6-Tetraethylenimino- 1,4-benzoquinone (4239-06-9)	Vogel and Leigh, 1975	M	A	F	Spm	1 mM	2
1-Phenyl-3,3-dimethyl- triazine (7227-91-0)	Vogel, 1973	M	A	F	Spm	1 mM	2
	Vogel et al., 1973	M	A	F	Spm	3.3 mM	2
Arsenic (7440-38-2)	Dugatova et al., 1978	M	A	F	NI	0.1%	3

TABLE 3 (continued)

Chemical (CAS No.)	References	Treatment parameters					Comments ^e
		Sex ^a	Stage ^b	Route ^c	Cell ^d	Concentration	
Hydroxylamine (7803-49-8)	Fahmy and Fahmy, 1970	M	A	IJ	NH	5-125 mM	3
Calcium chromate (13765-19-0)	Zimmering, 1983b	M	A	F	Spm	0.5 mg/ml	2
1-(3-Pyridyl-N-oxide)- 3,3-dimethyltriazine (21600-42-0)	Vogel et al., 1973	M	A	F	Spm	3.3 mM	2
Trophosphamide (22089-22-1)	Vogel, 1975	M	A	F	Spm	10 mM	2
α -Amanitin (23109-05-9)	Mollett and Skripsky, 1974	M	A	IJ	Spm	11 μ m	1,2
Hycanthon methane- sulfonate (23255-93-8)	Kramers, 1981	M	A	IJ	Spm, Tid	4.4 mM	2
	Zimmering, 1983b	M	A	F	Spm	10 mg/ml	1, 2
Butylated hydroxyanisole (25013-16-5)	Prasad and Kamra, 1974	M	A	IJ	Spm	10 ppm	1, 2
Acetin (26446-35-5)	Zimmering, 1982a	M	A	F	Spm	50 mg/ml	2
1-(2,4,6-Trichlorophenyl)- 3,3-dimethyltriazine (50355-74-3)	Vogel and Leigh, 1975	M	A	F	Spm	0.2 mM	2
	Buchi and Burki, 1975	M	A	F	Spm	5.5 mM	2
	Buchi, 1977	M	A	F	Spm	3.45-6.9 mM	2, 3

^a See Table 2. ^b See Table 2. ^c See Table 2. ^d See Table 2. ^e Reasons for inconclusive result given in Table 2. ^f Vapor.

formed. Many of the reviewed experiments were designed to assay for sex chromosome loss from postmeiotic germ cells treated with clastogenic agents. As suggested above, considering the possibility that apparent chromosome loss could not be distinguished from gross deletions in these experiments, it was decided that data from postmeiotic germ cells could not be used as evidence for aneuploidy induction and chromosome loss data could not be used without supporting evidence.

In all cases the reviewed data were examined for statistical significance, using the Normal test described by Margolin et al. (1983). Positive evidence for nondisjunction almost always could be accepted when the appropriate germ cells were sampled. Statistically negative results are the most difficult to classify and to evaluate. The minimum requirement for acceptability of a negative result was that appropriate germ cell stage(s) be ade-

quately sampled. In practice, this meant that the sample sizes both of the treated and control series were sufficient to have detected an increase of 0.2% in the aneuploidy frequency with a power of 75%. If the germ cell stage could not be determined with any certainty, the results were considered inconclusive.

The statistical classifications are as follows:

+ = a statistically significant difference at the 5% level between the treated and control frequencies.

- = no significant difference at the 5% level when the number of offspring tested was sufficient to have identified an increase of 0.2% over the control with a power of 75%.

I = inconclusive. No significant difference at the 5% level but the number of offspring tested was below that necessary to detect an increase of 0.2% with a power of 75%. Also, this category was

used when the interpretation of the data was questionable for any of the following reasons: (a) inadequate sample size to determine a negative or a positive, (b) the nature of apparent complete loss is undetermined, or (c) the nature of the germ cells sampled is not determined.

(5) Test performance

Of the 76 compounds analyzed, judgments regarding the ability to induce aneuploidy were made on 34 compounds. 17/34 compounds were judged positive for chromosome gain (11/34 for chromosome gain and chromosome loss, 6/34 for chromosome gain only). Similarly, 17/34 compounds were judged negative for chromosome gain (11/34 for chromosome gain and chromosome loss and 6 for chromosome gain only). A question of interest is the following: Are any of the compounds found to induce aneuploidy capable of inducing gene mutations and/or chromosome breakage in *Drosophila*? (i.e., are any specifically aneuploid inducers?). Table 4 provides related in-

TABLE 4
RESPONSE OF COMPOUNDS IN TESTS FOR GENE MUTATION AND/OR CHROMOSOME BREAKAGE THAT WERE POSITIVE IN TESTS FOR ANEUPLOIDY (CHROMOSOME GAIN)^a

Compound	Chromosome gain	Other tests ^b
<i>p,p'</i> -DDT	+	+
Actinomycin-D	+	0
5-Fluorodeoxyuridine	+	0
Caffeine	+	+
MNNG	+	+
Acenaphthene	+	0
Proflavine	+	0
1,2-Dichloroethane	+	+
Sodium cyclamate	+	0
Halothane	+	+
Azathioprine	+	+
Colcemid	+	0
Vinblastine	+	0
Methylmercury hydroxide	+	0
Clophen A50	+	0
Bleomycin	+	+
Satratoxin-H	+	0

^a +, positive; 0, no data or inconclusive.

^b Implies tests for gene mutation and/or chromosome breakage.

formation based on the EPA Gene-Tox reports on recessive lethals (Lee et al., 1983) and chromosome mutations (Valencia et al., 1984) for the 17 compounds positive for chromosome gain in the present review.

Since 7 of the compounds positive for chromosome gain were positive in one or more other tests and no reliable data exist regarding results in other tests for the remaining 10 compounds, the answer to the question awaits results from further work.

(6) Strengths and weaknesses of the assay systems

The panel considers it premature to discuss strengths and weaknesses of aneuploidy test systems in *Drosophila* since commonly accepted standards for the validation of a screen have not yet been met, e.g. (1) repeatability of results within a given laboratory in tests with a large number of compounds, (2) agreement between different laboratories testing a common set of chemicals with the same protocol, and (3) an acceptable level of concordance with test systems in other organisms. Further, it is likely that aneuploidy screening in the future will make use of special selective genetic systems permitting far larger sample sizes to be obtained and providing a firmer data base for judgments regarding the aneuploidy-inducing ability of tested chemicals.

(7) Method development

Several selective detection schemes to monitor for aneuploidy induction in the female *Drosophila* are under development; these are described briefly below.

(1) The scheme of P.D. Smith (1983) is designed to detect chromosome gain only. It makes use of the observation that larvae carrying X-linked *mal* (maroon-like) mutations are deficient for xanthine dehydrogenase (XDH) and, therefore, killed in the presence of a high concentration of purine. Essentially, females heterozygous for two different complementing *mal* mutations (e.g., *mal*¹ + / + *mal*²) are treated as larvae or adults and mated with males carrying the equivalent of *mal*¹ *mal*². F₁ larvae are treated with high purine concentrations killing the *mal*¹ and *mal*² regular males

and the $mal^1 mal^2$ exceptional males; survivors are the $mal^1 +/+ mal^2$ /exceptional females. Counts of eggs or of progeny from bottles not treated with purine are used to estimate sample size.

(2) The translocation XY system (Craymer, 1974; Foureman, 1979) monitors chromosome gain only in females. Females homozygous for $y w^a$ are mated with males carrying a translocation between the X and the doubly marked Y chromosome $B^S Y y^+$. The translocation breakpoint is in the middle of the X chromosome; the distal segment of the X, X^D , is marked with y^+ , and the proximal segment, X^P , with B^S . X^D and X^P behave as a bivalent and segregate at meiosis I in the male. All regular offspring die (X/X^D and X/X^P). Nondisjunction in the female produces diplo-X and nullo-X eggs. The former are recovered as a hyperploid female (XX/X^D or XX/X^P) and phenotypically w^a or $y w^a B^S$; the latter are not recoverable. Sample size is based on the number of exceptional females in controls.

(3) In the scheme of R. Grell (1981), females carrying normally arranged X chromosomes heterozygous for closely linked lethals at the far proximal end of the chromosome are mated with appropriately marked males. Specifically, $y w^a f l^1 +/y w^a f + l^2$ females are mated with $y^2 v B/y^+ Y$ males. All regular males die with the exception of crossovers between the lethals. Regular females are phenotypically $y^2 v B/+$, exceptional females are $w^a f$ and exceptional males $y^2 v B$. Paternal nondisjunction produces phenotypically B females and lethal males.

Independently (Zimmering, unpublished observation), a scheme employing heterozygous lethals in heterozygously inverted X chromosomes was designed. The system is referred to as 'FIX' (free inverted X chromosomes). Females homozygous for y , heterozygous for the complex sc^8 In49 inverted X and heterozygous for two X chromosome lethals (one in each X) are mated with attached-XY w males not carrying a free Y chromosome. All regular males die (except for occasional double crossovers permitting survival of regular males). Regular females are phenotypically wild-type, exceptional females y and exceptional males w .

(4) The 'CDX' system (Zimmering, unpub-

lished observation) is a variation of the lethal-bearing systems described above. The Muller $C(1)DX$ compound X chromosome is deficient for *bobbed*. Individuals not carrying a normal allele of *bobbed* die. CDX females of the composition $y w f bb^-/y^+ Y$ are mated with y attached-XY males not carrying a free Y chromosome. Regular females die, regular males are phenotypically y , exceptional females w , and exceptional males, wild-type. In the Grell and Zimmering schemes, half the regular progeny die, and the remainder are retained for statistical analysis.

(5) The *zeste* scheme (Zimmering, unpublished observation) is a selective free-X scheme carrying no lethals (except for those arising spontaneously) and designed to permit the recovery of all exceptional females, i.e., those recovered from (a) no-exchange tetrads, (b) equational exceptions arising following exchange and nondisjunction, and (c) meiosis II nondisjunctions resulting from failures of sister-chromatid separation. The scheme takes advantage of the sexual dimorphism of the *zeste* (z) mutation; hemizygous z males are phenotypically wild-type, whereas, homozygous z females have apricot-colored eyes. Homozygous $y z$ females are mated with $y^2 z$ males and the $F_1 y z/y^2 z$ females mated with attached-XY w males not carrying a free Y chromosome. Regular females and males are phenotypically wild-type, exceptional females are z and y^2 or y , the latter a recognizable product of events (b) or (c) described above, and exceptional males are w . Accordingly, scoring for exceptionals is as rapid and efficient as in the 'FIX' and 'CDX' systems.

Judgments regarding the selection of a preferred genetic scheme (or schemes) would take into account the following features: (1) chromosome gain and chromosome loss can be monitored, (2) exceptionals are identified rapidly and efficiently, (3) a reliable data base for statistical analysis is established, (4) the genetic scheme is arranged so that P_1 females are automatic virgins, (5) exceptional females derived from the several different kinds of events described above are recoverable, and (6) all flies have good viability. Genetic schemes possessing all (or most) of the above features could then undergo validation studies and one or more could be adopted for use if they meet the criteria for validation described above.

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